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(54) Food or pet food composition containing plant extracts for maintenance of bone health and prevention or treatment of bone diseases

(57) The present invention relates to a composition for maintenance of bone health or prevention, alleviation and/or treatment of bone disorders. It also relates to the use of the composition in the manufacture of a nutritional product, a supplement or a medicament; and a method

of promoting bone growth or for the maintenance of bone health which comprises administering an effective amount of the composition.

Detailed Description of the Invention

[0016] With respect to the first object of the present invention, the plant or plant extract is selected from the group consisting of *Taraxacum* and *Amelanchier*.

[0017] In a preferred embodiment, the plant or plant extract is *Taraxacum officinale* (common dandelion), *Taraxacum kok-saghyz* or *Amelanchier ovalis*, *Amelanchier alnifolia*, *Amelanchier laevis*, *Amelanchier arborea*, *Amelanchier asiatica*, for example.

[0018] The plant according to the invention may be from any part of the plant source, e.g. leaves, tubers, fruit or roots. In a most preferred embodiment, leaves or roots of *Taraxacum* species, or fruits of *Amelanchier* species, or a mixture thereof are used. The plant or plant extract may be in the form of a dried, lyophilized extract of leaves, roots and/or fruits depending on the source of plant, or fresh plant, or enriched fraction obtained by inorganic or organic solvent extraction process known in the art.

[0019] The plant or plant extract according to the invention may be used in the preparation of a food composition. The said composition may be in the form of a nutritionally balanced food or pet food, a dietary supplement, a treat or a pharmaceutical composition.

[0020] The plant or plant extract may be used alone or in association with other plants such as chicory, tea, cocoa, or with other bioactive molecule such as antioxidants, fatty acids, prebiotic fibers, glucosamine, chondroitin sulphate, for example.

[0021] In one embodiment, a food composition for human consumption is prepared. This composition may be a nutritional complete formula, a dairy product, a chilled or shelf stable beverage, soup, a dietary supplement, a meal replacement, and a nutritional bar or a confectionery.

[0022] Apart from the plant extract according to the invention, the nutritional formula may comprise a source of protein. Dietary proteins are preferably used as a source of protein. The dietary proteins may be any suitable dietary protein; for example animal proteins (such as milk proteins, meat proteins and egg proteins); vegetable proteins (such as soy protein, wheat protein, rice protein, and pea protein); mixtures of free amino acids; or combinations thereof. Milk proteins such as casein, whey proteins and soy proteins are particularly preferred. The composition may also contain a source of carbohydrates and a source of fat.

[0023] If the nutritional formula includes a fat source, the fat source preferably provides about 5% to about 55% of the energy of the nutritional formula; for example about 20% to about 50% of the energy. The lipids making up the fat source may be any suitable fat or fat mixtures. Vegetable fats are particularly suitable; for example soy oil, palm oil, coconut oil, safflower oil, sunflower oil, corn oil, canola oil, lecithins, and the like. Animal fats such as milk fats may also be added as desired.

[0024] A source of carbohydrate may be added to the

nutritional formula. It preferably provides about 40% to about 80% of the energy of the nutritional composition. Any suitable carbohydrates may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, and maltodextrins, and mixtures thereof. Dietary fibre

5 may also be added if desired. If used, it preferably comprises up to about 5% of the energy of the nutritional formula. The dietary fibre may be from any suitable origin, including for example soy, pea, oat, pectin, guar gum, gum arabic, and fructooligosaccharides. Suitable vitamins and minerals may be included in the nutritional formula in an amount to meet the appropriate guidelines.

[0025] One or more food grade emulsifiers may be incorporated into the nutritional formula if desired; for example diacetlyl tartaric acid esters of mono- and di-glycerides, lecithin and mono- and di-glycerides. Similarly suitable salts and stabilisers may be included. Vitamins and minerals may also be combined with the plant extract.

[0026] The nutritional formula is preferably enteraly administrable; for example in the form of a powder, tablet, capsule, a liquid concentrate, solid product or a ready-to-drink beverage. If it is desired to produce a powdered nutritional formula, the homogenised mixture is transferred to a suitable drying apparatus such as a spray drier or freeze drier and converted to powder.

[0027] In another embodiment, a nutritional composition comprises a milk based cereal together with a prebiotic formulation. Preferably the milk based cereal is an infant cereal which acts as a carrier for the prebiotic formulation.

[0028] In another embodiment, a usual food product may be enriched with at least one plant or plant extract according to the present invention. For example, a fermented milk, a yoghurt, a fresh cheese, a renneted milk, article of confectionery, for example a sweet or sweetened beverage, a confectionery bar, breakfast cereal flakes or bars, drinks, milk powders, soy-based products, non-milk fermented products or nutritional supplements for clinical nutrition.

[0029] The amount of the plant or plant extract in the composition may vary according to the plant source and its utilization. In a preferred embodiment, an efficient daily dose amount is of at least about 1 mg, and more preferably from 1 mg to 200mg of the active molecule per day.

[0030] Also, the plant or plant extract according to the invention may be used in the preparation of a pet food composition. The said composition may be administered to the pet as a supplement to its normal diet or as a component of a nutritionally complete pet food, and more preferably in an hypocaloric pet food. It may also be a pharmaceutical composition.

[0031] Preferably, the pet food composition contains about 0.01 to 0.5 g of dry plants per gram of dry pet food for a 15 kg dog; and 0.001 to 0.1 g of dry plants per gram of wet pet food for a 15 kg dog.

Materials and methods

Preparation of extracts for screening assays:

[0044] The ground plant material is defatted with hexane then extracted with a mixture of alcohol and water, with different percentages of water from 10 to 90%, preferably with 50%. The alcohols can be methyl or ethyl alcohols, giving the extract 1a.

[0045] On an aliquot of the residue of this first extract, an enzymatic hydrolysis is carried out with a and b glucosidases. Enzymes can be replaced by acidic conditions. The operation may be done under mild conditions (room temperature) or through reflux with different acid concentrations. The aqueous hydrolysed phase is extracted with a non-miscible solvent, preferably ethylacetate to give the extract 2a.

[0046] The extract can be dried, freeze-dried or supplied as a liquid form.

In some cases, polyphenols can be discarded through a polyvinylpolypyrrolidone (PVPP) treatment, avoiding artefact with the screening assays.

[0047] Following the extract preparation, each extract was weighed, redissolved in dimethylsulphoxide (DMSO) to a final concentration of 20 mg/ml and stored in aliquots at -20°C. This was used as a stock solution and was subsequently diluted in media for each assay. A range of doses was tested in the assay systems.

[0048] Extracts of *Amelanchier ovalis* (P.E. 219 (MeOH/water)), and of *Taraxacum officinale* (P.E. 750 (ethylacetate)) were further tested in a human periosteal/pre-osteoblast cell line, hPOB-tert for their ability to induce the endogenous expression of BMP-2.

[0049] The validation of this assay was performed with statins as a positive control. At confluence, cells were incubated with 0.05 mg/ml Lovastatin or with the plant extracts. Total RNA was extracted with TRIzol Reagent (Gibco), 10 µg RNA were reverse transcribed using the 1st Strand cDNA Synthesis Kit (Boehringer). BMP-2 cDNA sequences were amplified for 35 cycles at an annealing temperature of 55°C using specific oligonucleotide primers (5':TTGCCGCTGCTCAGCAT-GTT; 3':CATCTTCATCTGTTCTCGGAA). PCR products were separated by agarose gel electrophoresis and detected by ethidium bromide staining. Quantification was performed using NIH Image Software and normalizing results with Actin as housekeeping gene.

Results :

[0050] For *Amelanchier ovalis* : induction of BMP-2 by 2.5 fold.

For *Taraxacum officinale* induction of BMP-2 by 2.0 fold.

Example 2 : Effect of *Amelanchier* and *Taraxacum* species on bone resorption

[0051] The ability of the extracts prepared as in ex-

ample 1, to inhibit IL-1 (10^{-10} M) stimulated bone resorption was assessed in the neonatal bone resorption assay. Each extract was assessed for its capacity to inhibit bone resorption at 10 µg/ml

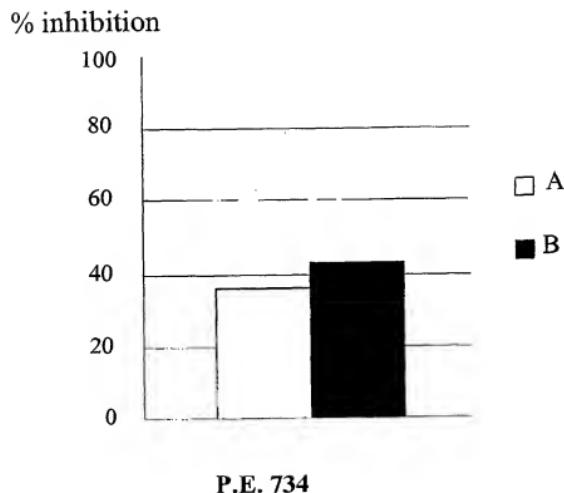
- 5 [0052] Extracts of fruits of *Amelanchier alnifolia* (10 µg/ml) (P.E. 734 (ethylacetate)) were able to inhibit IL-1 induced bone resorption in the murine calvaria assay (R.J. Murills, "In vitro Bone Resorption Assays" in Principles of Bone Biology (Academic Press) 1986, chap. 90). This effect was confirmed in a second bone resorption test, namely the pit assay using rabbit bone mixed cell cultures on bovine bone slices (Tezuka K., et al., 1992, *Biochem. Biophys. Res. Commun.*, 186(2):911-7 and Lorget F., et al., 2000, *Biochem. Biophys. Res. Commun.*, 268(3):899-903). Resorption pits are visualized by staining for TRAP (tartrate resistant acid phosphatase) positive cells and counted.
- 10 [0053] A comparison of activity of the extracts at 10 µg/ml in the two assay systems is shown in Figure 1.
- 15 [0054] A feed mixture is made up of about 58% by weight of corn, about 5.5% by weight of corn gluten, about 22% by weight of chicken meal, 2.5% dried chicory, about 10% of extract of *Taraxacum* leaves, salts, vitamins and minerals making up the remainder.

- 20 [0055] The feed mixture is fed into a preconditioner and moistened. The moistened feed is then fed into an extruder-cooker and gelatinised. The gelatinised matrix leaving the extruder is forced through a die and extruded. The extrudate is cut into pieces suitable for feeding to dogs, dried at about 110°C for about 20 minutes, and cooled to form pellets.
- 25 [0056] This dry dog food has a positive effect on bone and cartilage health and increase their mobility.

Claims

- 40 1. A food composition intended for the prevention, alleviation and/or treatment of bone disorders or maintenance of bone health in humans and pets, which comprises as an active ingredient an effective amount of at least one plant or plant extract selected from the group consisting of *Taraxacum* and *Amelanchier*.
- 45 2. A composition according to claim 1, wherein the plant or plant extract is selected from the group consisting of *Taraxacum officinale* (common dandelion), *Taraxacum kok-saghyz*, *Amelanchier ovalis*, *Amelanchier alnifolia*, *Amelanchier laevis*, *Amelanchier arborea* and *Amelanchier asiatica*.
- 50 3. A composition according to claims 1 or 2, wherein the plant or plant extract is from leaves or roots of *Taraxacum* species, or fruits of *Amelanchier spe-*

FIGURE 1





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INCOMPLETE SEARCH
SHEET C

Application Number

EP 01 20 4839

Although claims 14-19 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) searched completely:
1-13

Claim(s) searched incompletely:
14-19

Reason for the limitation of the search (non-patentable invention(s)):

Article 52 (4) EPC - Method for treatment of the human or animal body by therapy

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 01 20 4839

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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08-05-2002

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